Session I - Membrane Protein Crystallization I

Sunday, July 3 - morning

S1-L1

AN X-RAY LASER STRUCTURE OF RHODOPSIN-ARRESTIN COMPLEX

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Serial femtosecond X-ray crystallography (SFX) using an X-ray free electron laser (XFEL) is a recent advancement in structural biology for solving crystal structures of challenging membrane proteins, including G-protein coupled receptors (GPCRs), which often only produce microcrystals. XFEL delivers highly intense X-ray pulses of femtosecond duration short enough to enable the collection of single diffraction images before significant radiation damage to crystals sets in. In this talk, I will present our efforts toward the first structure of arrestin bound to rhodopsin - a prototype of GPCR, including strategies, pitfalls and successes in crystallizing the rhodopsin-arrestin complex as well as methods of XFEL data collection and analysis, structure determination, and the validation of the structural model. The rhodopsin-arrestin crystal structure solved with SFX represents the first near-atomic resolution structure of a GPCR-arrestin complex, provides structural insights into understanding of arrestin-mediated GPCR signaling, and demonstrates the great potential of this SFX-XFEL technology for accelerating crystal structure determination of challenging proteins and protein complexes.

S1-L2

ENGINEERING HETEROTRIMERIC G PROTEINS TO FACILITATE CRYSTALLISATION OF GPCRs IN THEIR ACTIVE CONFORMATION

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G protein-coupled receptors (GPCRs) are integral membrane proteins that regulate cytoplasmic signalling, through heterotrimeric G proteins and -arrestins. Structure determination of GPCRs in all activation states is vital to elucidate the precise mechanism of signal transduction, and to facilitate optimal drug design. However, due to their inherent instability, crystallisation of GPCR–G protein complexes has proved particularly challenging.

We used rational design mutagenesis to develop a minimal G protein, mini-G_s, which is composed of a single domain from the adenylate cyclase stimulating G protein (G_s). Mini-G_s induces similar pharmacological and structural changes in GPCRs as the heterotrimeric G protein, but eliminates many of the problems associated with crystallisation of these complexes, specifically their large size, conformational dynamics and instability in detergent.

We have determined the structure of the wild type human adenosine A_{2A} receptor ($A_{2A}R$) bound to mini-G_s at 3.4Å resolution by X-ray crystallography. The structure revealed large, mutually induced conformational changes in both the receptor and G protein, and has provided unique insight into the mechanism of GPCR activation.

S1-L3

NEW AND IMPROVED FEATURES OF THE LIPID CUBIC PHASE (*IN MESO*) METHOD FOR CRYSTALLIZING MEMBRANE AND SOLUBLE PROTEINS AND COMPLEXES

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The lipid cubic phase (*in meso*) method for crystallizing membrane proteins has been in use now for two decades (1). In part because the cubic mesophase in which crystals grow is extremely viscous and sticky, the method in its infancy was adopted with great hesitancy and only by the truly adventurous. As the structures to emerge from the method grew in number and profile, so too did interest in the method (2). This was due in no small measure to advances in making the method more efficacious, versatile, user-friendly and high-throughput. Advances continue to be made and three of the more recent ones will be summarized in this presentation. The first addresses the many challenges faced by needing to open the glass sandwich plate used for crystallization in order to harvest crystals. This led to the *in meso in situ* serial crystallography (IMISX) method which enables data collection in the mesophase where and as crystals grow without the need to directly harvest crystals (3). It is a simple, robot-compatible method that requires miniscule (ng – single digit g) amounts of protein and can be used for data collection at room and at cryo temperatures. A distinctly different '*in situ'* approach has proven successful in the area of serial femtosecond crystallography. In this case, crystals grown in the cubic mesophase are extruded across a micrometer-sized free-electron laser X-ray beam for data collection at ambient temperature, again without the need for direct crystal harvesting (4). The third advance refers to unstable proteins that cannot be concentrated in functional form by standard methods to values required for crystallization. The new Cubicon method solves this problem by using the cubic mesophase itself as a medium in which to concentrate the membrane protein through sequential rounds of reconstitution for direct use in *in meso* crystallization trials.

These few examples bear witness to the fact that the *in meso* method, whilst mature and robust, continues to evolve and to improve. It has a bright future and not only in its application to membrane proteins. Increasing attention is being paid to the method and to the mesophase itself for use with soluble proteins (2).

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S1-L4

THE IMPORTANCE OF DETERGENT SELECTION: ARE YOU CHOOSING THE RIGHT DETERGENT FOR YOUR MEMBRANE PROTEIN?

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In the time that has elapsed since the deposition of the first membrane protein structure in 1985, we have witnessed many significant strides in the field. Despite the substantial growth and tremendous success, bottlenecks persist throughout the membrane protein workflow, including expression, solubilization, purification and crystallization. For just as long, Anatrace Products has been a trusted resource in membrane protein research, due to their large portfolio of detergents and lipids, and unsurpassable standards in quality and reproducibility.

We are excited to present an updated overview of detergent usage in membrane protein solubilization, purification, and crystallization which highlights the importance of detergent choice throughout each step of the membrane protein crystallization pipeline. Always committed to innovating, we have developed new tools to simplify the detergent selection process. Data will presented from our Analytic Extractor and Analytic Selector kits, designed to facilitate the choice of detergent for protein extraction (Extractor) and downstream applications including crystallization, NMR, EM, and binding studies (Selector). Lastly, we will introduce new detergent offerings, including novel trehalose-based detergents, and bicelle kits for crystallization.

Session II - Crystallization of Macromolecular Complexes

Sunday, July 3 - afternoon

S2-L1

GENERATION OF ANTIBODY-Fab REAGENTS TO CAPTURE AND STABILIZE FUNCTIONALLY IMPORTANT CONFORMATIONAL STATES OF PROTEINS TO FACILITATE THEIR STRUCTURE DETERMINATION BY CRYSTALLOGRAPHY AND SINGLE PARTICLE Cryo-EM

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A high throughput phage display pipeline has been established to generate Fab-based synthetic antibodies that have been successfully used as high performance crystallization chaperones. The phage selection strategies have been developed to target Fab binding to specific surface epitopes or to capture and stabilize functional conformational states to facilitate probing both the protein's static and dynamic features, as well as the transitions between states. In most cases, the high throughput capability of the pipeline provides researchers with at least 10 unique Fabs that perform a desired function. A further enhancement for the generation of Fabs for membrane proteins is the use of lipid-filled nanodiscs to provide membrane- like environments for membrane proteins during phage display selections. Fabs generated from nanodisc formats provide guidelines for which detergents best (and worst) mimic the protein's membrane-like lipid environment, which can guide choices for setting up crystallization and Cryo-EM experiments.